IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of A. K. Gunnar Aberg.

Serial: 09/895,463

For: "TOLTERODINE METABOLITES"

DECLARATION UNDER 37 C.F.R. § 1.132

The Honorable Commissioner
Of Patents & Trademarks
Washington, D.C. 20231

Sir:

I, A.K. Gunnar Aberg, declare:

THAT I am a citizen of Sweden, a permanent resident of the USA, and resident of the City of Sarasota, Sarasota County, Florida;

THAT I am Chief Executive Officer of BRIDGE PHARMA, Inc., 902 Contento Street, Sarasota, Florida 34242. From 1968 to 1973, I was Director of Pharmacology at Bofors Nobel-Pharma (Sweden); from 1974 to 1978, I was Group Leader in General Pharmacology at AB Hässle (Sweden); from 1978 to 1980, I was Director of Pharmacology at Astra (USA); from 1980 to 1982, I was Director of Cardiovascular Pharmacology at Ciba-Geigy (USA); from 1982 to 1992, I was Director and Executive Director of Pharmacology at Squibb and Bristol-Myers Squibb; and from 1992 to 1996, I was Vice President and Senior Vice President of Research at Sepracor Inc. and I founded BRIDGE PHARMA, Inc. in 1996;

THAT I am a graduate of the University of Linköping, Sweden from which I hold a Ph.D. degree in Pharmacology and of the University of Gothenburg, Sweden from which I hold a degree in Zoophysiology, and that I am a docent (Associate Professor) in Applied Pharmacology at the University of Linköping, Sweden;

THAT I have over thirty years of pharmaceutical research experience;

THAT I am an author of over one hundred publications on pharmacological topics, including twenty publications on relaxation of smooth muscle, 16 publications on antiarrhythmics, and publications on urinary incontinence and calcium antagonists.

THAT I have made numerous scientific presentations on the subjects of smooth muscle relaxation and urinary incontinence;

THAT my thesis in pharmacology concerned vascular smooth muscle pharmacology and that my thesis in zoophysiology concerned intestinal smooth muscle physiology;

THAT I am an inventor of forty-three US patents and several pending patent applications, including the present Patent Application;

THAT I have reviewed the Office Action dated September 29, 2003 in the above referenced Application. I am also familiar with the application in the present case and the art cited by the Examiner, namely U.S. Patent No. 5,559,269 and U.S. Patent No. 5,686,46

THAT studies have been designed by me and performed under my close supervision and that the results support the subject application.

A summary of the Project Background, the Biological Study Results, and my Conclusions thereof will be presented here.

Declaration regarding U.S. Patent Appln. Serial No. 09/895,463

Background of Project:

An estimated 17 million people in the USA suffer from urinary urge incontinence. This corresponds to an estimated 50 million people worldwide. Two anticholinergic drugs dominate the market: oxybutynin (Ditropan®, ALZA/J&J) and tolterodine (Detrol®, Pharmacia/Pfizer). A third drug, terodiline (Micturin®, Kabi) was withdrawn from the market, since it was found to cause prolongation of the QTc-segment of the ECG, which indicates a very high risk for the development of a fatal type of cardiac arrhythmias that is called Torsades de Pointes. Both oxybutynin and tolterodine have now been found to cause the same type of QTc-prolongation that forced the withdrawal of terodiline. A withdrawal of either or both of oxybutynin and tolterodine may prove disastrous for all the patients who are depending on these drugs. A race is therefore ongoing in the pharmaceutical industry to find replacement therapy for oxybutynin and tolterodine.

We have unexpectedly found that a secondary amine metabolite of tolterodine (DES-TOLT) that previously was believed to be therapeutically inactive, actually potently inhibits the involuntary contractions of the urinary bladder that cause urinary urge incontinence. Furthermore, we have found this metabolite to be free from prolongation of the QTc interval of the ECG. Another major metabolite of tolterodine (5-HM or 5HM-TOLT) is already known to have therapeutic activity. We have unexpectedly found that also 5HM-TOLT is free from the much-feared side effect of QTc-prolongation.

Thus, the present patent application for DES-TOLT and 5-HM-TOLT concerns two different metabolites of tolterodine (TOLT). TOLT is an R-isomer and the corresponding racemate is here called RS-TOLT.

The first metabolite is:

5HM, (or 5HM-TOLT) which is the 5-hydroxymethyl metabolite of TOLT, and which chemically is R-(+)-N,N-diisopropyl-3-(2-hydroxy-5-(hydroxymethyl)phenyl)-3-phenyl-propylamine
and
RS-5HM, (or RS-5HM-TOLT) which is the racemic 5-hydroxymethyl metabolite of RS-TOLT, and which chemically is RS-N,N-diisopropyl-3-(2-hydroxy-5-(hydroxymethyl) phenyl)-3-phenylpropylamine.

Both 5HM and RS-5HM have been described and claimed in USP 5,559,269 and USP 5,686,464 by Johansson et al.

The second metabolite is:

DES-TOLT, which is secondary amine metabolite of TOLT, and which chemically is called R(+)-N-Isopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropylamine
and
RS-DES-TOLT, which is the racemic secondary amine metabolite of RS-TOLT, and which is called RS-*N*-Isopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropylamine.

DES-TOLT and RS-DES-TOLT have not previously been claimed in any patents, but are known metabolites of TOLT. DES-TOLT and RS-DES-TOLT have previously been considered as inactive, but have been found by us to be pharmacologically active and cause relaxation of contracted smooth muscle and prevent contractions of hyperactive smooth muscle *in vivo*.

INVENTIVE STEPS:

(1) The metabolite DES-TOLT (and RS-DES-TOLT) has previously been considered as inactive, but has now surprisingly been found by us to be pharmacologically active and potently cause relaxation of contracted smooth muscle and prevent contractions of hyperactive smooth muscle *in vivo*. The finding is surprising because all marketed drugs for urinary urge incontinence are anticholinergic (antimuscarinic) compounds, while des-tolterodine is devoid of antimuscarinic activity. The mechanism of the therapeutic action of DES-TOLT is not known, but there are several possibilities, including potassium channel activation, calcium channel inhibition, interaction with serotonin and numerous other mechanisms.

(2) There has not been any incentive to develop any of said metabolites (DES-TOLT and 5-HM-TOLT) into a new drug, since no advantages over the present therapy have been identified (the present therapy being TOLT = tolterodine = Detrol®, Pharmacia.) Since the incontinence drug TOLT has recently been found to cause prolongation of the QTc segment of the ECG and since QTc-prolongation is the most common cause of a fatal cardiac arrhythmia, called Torsades de Pointes, much work has been directed by us and others to finding non-arrhythmogenic replacement therapy for TOLT. The replacement for TOLT must cause relaxation of smooth muscle *in vivo* and must not cause any prolongation of QTc. The severity of the side effect called "Torsades de Pointes" is obvious from the fact that another incontinence drug (terodiline, Micturin®) as well as an antihistamine (terfenadine, Seldane®) and several other drugs, such as for example astemizole (Hismanal®), have been withdrawn from the market by regulatory authorities worldwide because these drugs caused prolongation of QTc. It is now feared that the incontinence drugs tolterodine and oxybutynin may be withdrawn from the market by the FDA and its international regulatory counterparts.

Interestingly and importantly, we have found that neither of the two therapeutically active metabolites of tolterodine (DES-TOLT and 5HM-TOLT) cause QTc-prolongation. Thus, the arrhythmogenic activity of TOLT resides in the parent compound (tolterodine "itself").

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For pharmacological testing, see next page.

PHARMACOLOGICAL STUDIES OF TWO TOLTERODINE METABOLITES

1. Ligand binding studies: Affinity for muscarinic receptors.

TEST METHOD

These experiments were carried out using human recombinant M1, M2 and M3 muscarinic receptor subtypes expressed in membranes of an insect cell line (SF9). The compounds were tested on each receptor at multiple concentrations in duplicate to obtain competition curves. In order to validate each experiment, a reference compound (tolterodine; TOLT) was tested simultaneously. The radioligand for M-1h was [3H]pirenzepine, 2 nM; for M-2h the ligand was [3H]AF-DX 384, 2 nM; and for M-3h the ligand was [3H]4-DAMP, 2 nM. Atropine (1 μM) was the nonspecific ligand in all experiments and all incubation times/temperatures were 60 min/27°C, respectively.

TEST RESULTS

The test results are shown in the following Table 1:

	Human Muscarinic Receptors (IC50; nM)		
Compound	M-1h	M-2h	M-3h
TOLT	4	6	13
5-HM-TOLT	2	4	2
DES-TOLT	63	>100	>100

M-1 muscarinic receptors are found for example in the eye and in salivary glands, and inhibition of M-1 receptors causes blurry vision, dry eyes and dry mouth.
M-2 and (particularly) M-3 receptors are found in the urinary bladder and in several other smooth muscles, such as for examples in the kidneys, in the intestines and in the gall bladder. Inhibition of M-2 and M-3 receptors offer therapeutic advantages for patients suffering from various types of smooth muscle hyperactivity, such as urinary urge incontinence, intestinal hyperactivity, and smooth muscle cramps caused by gall stones and kidney stones.
The terms "M-1h", "M-2h" and "M-3h" refer to human muscarinic receptors.

CONCLUSIONS

Previously known results concerning affinity of tolterodine (TOLT) and the 5-hydroxymethyl metabolite of tolterodine (5-HM-TOLT) were confirmed.
Des-tolterodine had no measurable affinity for M-2 and M-3 muscarinic receptors, leading to the conclusion that this compound (DES-TOLT) is pharmacologically inactive. Concomitant studies *in vivo* (see page 6 – 8) later proved that this conclusion was not correct under *in vivo* circumstances, but DES-TOLT proved to be active *in vivo*.

2. Functional *in vitro* studies: Anticholinergic effects

Smooth muscle relaxation has been studied using isolated strips from male rat urinary bladders. The purpose of these studies was to determine antimuscarinic activity in a functional test system.

TEST METHODS

Strips of tissue (≤ 10 mm long and 1.0 mm wide) were removed from the urinary bladder of male Sprague-Dawley rats, weighing 300 - 600 grams. The bladder strips – containing detrusor muscle tissue – were suspended in an oxygenated tissue bath medium of the following composition, in mmol/l: NaCl, 133; KCl, 4.7; CaCl2, 2.5; MgSO4, 0.6; NaH2PO4, 1.3; NaHCO3, 16.3; and glucose, 7.7. They were maintained at 37.5 °C. Contractions were recorded isometrically (Grass Instrument Division, Astro-Med, Inc., West Warwick, RI, USA) and a resting tension of 1.0 g was maintained. Up to 8 bladder strips of bladder were removed from a single bladder, suspended in individual tissue chambers and allowed to equilibrate with the bathing solution for one hour before proceeding with the experiment.

Contractions of each strip of tissue were recorded initially in response to cumulatively increasing concentrations of carbachol. One strip was kept untreated while the remaining strips were each exposed to one concentration of the test compound for 60 min, after which time the responses to increasing concentrations of carbachol were again recorded. The highest tension developed by each strip during the second set of concentration-effect determinations was expressed as a percentage of the maximum tension developed during the first set of such determinations. To quantitate the antimuscarinic action of the compounds, the program Prism (Graphpad, Inc.) was used to average the data from the experiments, create dose-response curves, and calculate the antimuscarinic activity of the test compounds.

TEST RESULTS

The following table summarizes the results from tests with carbachol-induced and potassium-induced contractions of rat bladder strips.

Table 2. Anticholinergic effects of TOLT metabolites (partial results)

Compound	Antagonism of contractions induced by carbachol (K_B (nM); n)
TOLT	2; 8
5-HM-TOLT	1; 8
DES-TOLT	84; 8

K_B = the molar concentration of an antagonist that doubles the EC_{50} of an agonist.
N= number of tests.

CONCLUSIONS

There was a good correlation between the results from muscarinic receptor binding studies and the functional smooth muscle relaxation studies.

Thus, as antimuscarinic agents, 5-HM-TOLT was more active than TOLT and DES-TOLT was practically inactive, leading to the conclusion that DES-TOLT is not pharmacologically or therapeutically active in patients suffering from smooth muscle hyperactivity conditions, such as for example urinary urge incontinence. Concomitant studies *in vivo* (see page 5 – 8) later proved that this conclusion was not correct under *in vivo* circumstances, but DES-TOLT proved to be active *in vivo*.

3. *In vivo* studies: Effects on hyperactive urinary bladder hyperactivity.

TEST METHODS

The test method used in the present *in vivo* evaluations measures spasmolytic activities of the test articles in the hyperactive urinary bladder of rats.

In these experiments, male Wistar-Kyoto rats (approx. 250g) were anesthetized with a single intraperitoneal dose of urethane (1.25 g/kg). The degree of anesthesia was monitored frequently; no additional dose of the anesthetic was ever needed. Body temperature was maintained using a warm water pad. The urinary bladder was exposed through a midline incision in the abdomen; both ureters were ligated and cut. A small incision was made at the top of the bladder and a balloon was inserted through the cut. Warm water of 37°C was injected into the balloon to obtain an increased internal pressure in the bladder of about 10 mm Hg, and the ensuing isovolumetric hyperactivity in the form of bladder contractions that were recorded using a pressure transducer. When the frequency of bladder contractions had reached a constant level (generally after a 60 min), a solution containing the test article was administered intravenously at a rate of 1 ml/kg over a period of 30 seconds. The effects upon the contractions of the bladder were measured continuously for 60 min. The test articles were dissolved in a vehicle consisting of 10% DMSO in saline.

TEST RESULTS

Graphs showing the test results are shown on the following pages.

Graph 1: There was no effect of the vehicle in this test system.

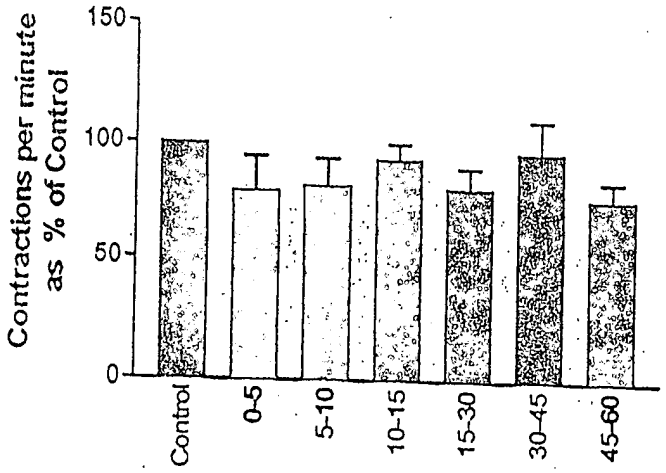
Graph 2: The effects of TOLT, 2.5 mg, were statistically significant.

Graph 3: The effects of DES-TOLT, 2.5 mg, were statistically significant.

CONCLUSIONS

The results obtained with the vehicle (saline) and tolterodine, confirm clinical results, thereby validating the test system. The results obtained with 5-HM-TOLT could be expected from the receptor binding studies and the functional antimuscarinic results. The positive results with DES-TOLT were unexpected since DES-TOLT does not have antimuscarinic activity.

Three graphs are shown on next page.



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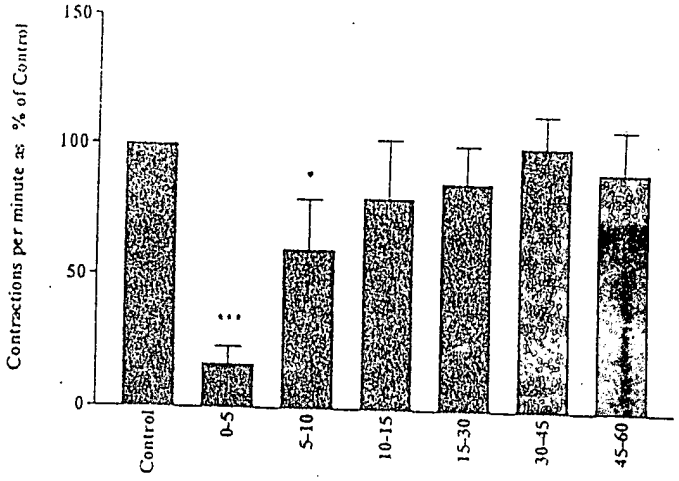
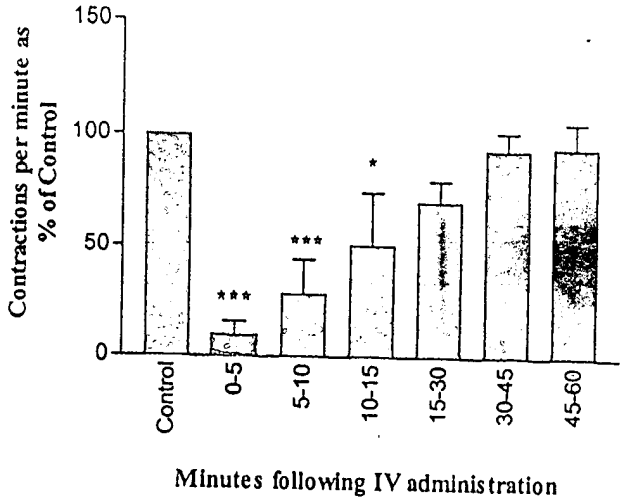


TOLT



DES-TOLT

4. *In vivo* studies: Arrhythmogenic activity

Terodiline was withdrawn from the market because the drug caused prolongation of the QTc interval of the ECG and concomitant Torsades de Pointes arrhythmias. It is therefore important to carefully investigate possible effects on the QTc interval by all new compounds that are even remotely related to the chemical structure of terodiline. Few studies are available, describing the effects of incontinence drugs on the cardiac action potential or the QT interval of the ECG by drugs other than terodiline. However, using electrophysiological methods, researchers have studied cardiac effects of RS- and S-oxybutynin *in vitro*. We have performed studies *in vivo* that are summarized below.

TEST METHODS

Urethane - anesthetized guinea pigs were given iv infusions of 5 mg/kg of the test article over 30 min. ECGs were recorded for 30 minutes prior to the infusion and for 20 minutes after the termination of the infusion. QT intervals were measured at pre-determined intervals. QTc values were calculated using Bassett's formula. The effects of the test articles on QTc at -20 min, -10 min and ± 0 min (before the infusion) were compared to +30 min, +40 min and +50 min (after infusion).

TEST RESULTS

Table 3. Prolongation of QTc interval (ΔQTc) of the ECG of anesthetized guinea pigs, presented as percent of the pre-drug duration of the QTc interval in each animal.

Compound	N	ΔQTc (%)
Vehicle	8	− 3 ± 1
Terodiline	8	+ 11 ± 1
RS-oxybutynin	7	+ 12 ± 2
TOLT	7	+ 16 ± 1
DES-TOLT	8	− 2 ± 1
5-HM-TOLT	8	− 2 ± 1

N indicates the number of animals in each group

CONCLUSION

The validity of the test method was demonstrated by showing that terodiline (but not the vehicle) caused prolongation of QTc. Both oxybutynin and tolterodine caused prolongation of QTc.
The secondary amine metabolite of tolterodine (DES-TOLT) and the 5-hydroxy-methyl metabolite (5-HM-TOLT) did not prolong the QTc interval.

GENERAL CONCLUSIONS FROM BIOLOGICAL TESTING.

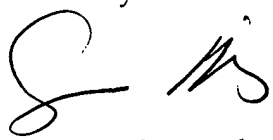
It was surprisingly found that the secondary amine metabolite of tolterodine (DES-TOLT) caused relaxation of the urinary bladder *in vivo*. This was totally unexpected since DES-TOLT did not express antimuscarinic activities in receptor binding tests or in functional anti-muscarinic studies. Previous compounds of the tolterodine class did not cause relief of urinary incontinence in patients and had no effects *in vivo* in relevant animal models unless they had antimuscarinic activity. The mechanism of the surprising therapeutic action of DES-TOLT is not known, but there are several possibilities, including potassium channel activation, calcium channel inhibition, interaction with serotonin and numerous other mechanisms.

A remarkable finding was that – contrary to tolterodine – both active metabolites of tolterodine (DES-TOLT and 5-HM-TOLT) were completely free from all effects on the QTc-interval of the ECG. It is therefore concluded that the increase in duration of the QTc-interval after administration of tolterodine is due to the parent compound (tolterodine "itself") and this activity is not residing in any of the two active metabolites. It is certainly unexpected that the 5-HM metabolite does not cause QTc-prolongation since there is only a very minor difference in chemical structure between 5-hydroxymethyl-tolterodine and tolterodine (that has a methyl group in this 5-position. In light of the fact that the severely arrhythmogenic drug terodiline is a secondary amine, it was also unexpected that the secondary amine metabolite (DES-TOLT) of tolterodine proved to be free from the much feared side effect of QTc-prolongation.

It should be added that the risk for Torsades de Point arrhythmias is further increased if the patient is using drugs like erythromycin or ketoconazole that are known to inhibit or delay the metabolism of drugs like tolterodine, terodiline, oxybutynin and terfenadine.

I further declare that all statements of the foregoing declaration made of my own knowledge are true and that those made upon information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that willful false statement may jeopardize the validity of the above-identified application or any patent issuing thereon.

Signed by me on this 13th day of December, 2003.



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